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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Martha K. Newell
Serial No.:	09/599,760
Filed:	June 22, 2000
For:	METHODS AND PRODUCTS FOR MANIPULATING UNCOUPLING PROTEIN EXPRESSION
Examiner:	Jane J. Zara
Art Unit:	1635

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Commissioner for Patents, Washington, D.C. 20231, on the 22 day of October, 2002.

Helen C. Lockhart

Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION OF DR. MARTHA KAREN NEWELL

I, Martha Karen Newell, Ph.D., declare as follows:

1. I make this declaration in support of U.S. Serial No. 09/599,760 on which I am named as the sole inventor.

2. I am a Professor of Biology at the University of Colorado in Colorado Springs. I have been performing research on uncoupling protein (UCP) expression since 1998. Prior to that time, I performed research in the field of immunology generally and, more specifically, to examine the mechanisms in MHC class II signaling and apoptosis and MHC class II mediated signaling and autoimmune disease.

3. I have performed several experiments in my laboratory which relate to my discovery of UCP in lysosomes, including examining the relationship between lysosomal pH, immune system responses and UCP inhibitors.

4. My invention is based at least in part on the finding that UCP inhibitors regulate lysosomal pH, and that the administration of UCP inhibitors is useful in the prevention or treatment of infectious diseases.

5. In order to demonstrate that active UCP in the lysosome regulates pH and that inhibition of UCP results in an enhanced immune response, UCP2 knock out mice were studied. These mice were obtained from Dr. Bradford Lowell at Harvard. We determined that mice lacking UCP2 exhibited both increased acidity in the lysosome (Exhibit 1) and statistically increased MHC class II expression (Exhibit 2) and increased numbers of B cells (Exhibit 3). We isolated splenocytes from wild type (C57Bl6.H129) mice and the strain matched UCP2 knockout animals. The cells were cultured for 24, 48, and 72 hours. Subsequent to culture, the cells were harvested, washed, and stained with Lysosensor Dyes (Molecular Probes, Inc.) to measure lysosomal pH. Lysosensor Dyes fluoresce as a function of increasing acidity in the lysosomes. As shown in Exhibit 1, the UCP2 knock out mice had more acidic lysosomes as a result of a decrease in lysosomal pH. We also stained cells for MHC class II expression. As shown in Exhibit 2 the knock out animals having a more acidic lysosomal compartment also expressed MHC class II at higher levels on the cell surfaces than control animals having functional lysosomal UCP. The knock out mice also demonstrated an increased number of MHC class II positive B cells (exhibit 3). Animals having increased expression of MHC class II on antigen presneting cells are considered to have enhanced ability to fight off infectious disease by mounting an antigen specific immune response. Thus, UCP in the lysosome regulates pH and inhibition of UCP results in an enhanced immune response

6. A recent article by Arsenijevic *et al.* (Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production, *Nature Genetics*, 2000,

26(4):387-8.) has shown that control mice (which are on a background strain) are highly susceptible to *Toxoplasma gondii*, and that the mice become extremely resistant without a functional UCP2 (i.e. UCP2 knock out mice). It is well established that the lysosome is centrally important in resistance to this organism. Arsenijevic *et al.* found that compared to wildtype mice, $UCP2^{-/-}$ (knockout) mice resisted and eliminated infectious challenge more efficiently.

7. Therefore, lysosomal UCP may be inhibited to regulate lysosomal pH and is useful to treat or prevent infectious disease.

8. I, Martha Karen Newell, Ph.D, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under §1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

October 4, 2002
DATE

Martha Karen Newell
MARTHA KAREN NEWELL, Ph.D